

Applicant: M. During
Serial No.: 09/491,896
Examiner: B. Bunner
Group Art Unit: 1647

--(triangle-solid line) --

At page 47, line 1, please delete "Neurobasal™" and replace with -- NEUROBASAL™ serum-free basal medium --

At page 49, line 26, please delete "Mini Complete™" and replace with -- MINI COMPLETE™ protease inhibitor composition—

At page 66, line 15, after "rats" please replace "(diamond-dashed line)" with --(triangle-solid line) --

Clean copies of the affected paragraphs are presented below followed by marked-up versions of the paragraphs.

Applicant: M. During
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Clean copy of the title at page 1

NMDA VACCINE FOR THE TREATMENT OF EPILEPSY

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Clean copy of the paragraph 5 at page 8

Fig. 7A is a graph showing the effect of vaccination of the behavior of rats in a line crossing test. Data represents the number of line crossings in 5 min intervals over 5 successive days in AAVlac-treated animals (squares-solid line) or AAVNMDAR1-vaccinated animals (triangle-solid line);

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Group Art Unit: 1647

Clean copy of paragraph 1 at page 47

NEUROBASAL™ serum-free basal medium containing B27 supplement and 0.5 mM L-glutamine (all from Gibco BRL). Medium was replenished every 48 h, with the addition of a mitotic inhibitor (0.5 μ M cytosine arabinoside) after 4 days. Cultures were grown for at least 9 days prior to calcium imaging.

Applicant: M. During
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Group Art Unit: 1647

Clean copy of paragraph 2 at page 49

For β -galactosidase antibody screening, 1 μ g purified β -galactosidase protein (Sigma) was separated on a 10% acrylamide gel under reducing conditions and transferred to a nitrocellulose membrane. Serum samples from AAVNMDAR1, naïve and AAVlac animals (1:200), or monoclonal β -galactosidase (1:5000, Gibco BRL) were applied for 1 h at room temperature (RT) or overnight at 4°C following a 90 min incubation in Tris-buffered saline containing 0.1% Tween 20 (TBST) containing 5% fetal calf serum (FCS) to block non-specific binding. Bound antibodies were detected using a peroxidase-labeled anti-rat or mouse antibody (1:12,000, Sigma) for 1 h at RT, and visualized using the ECL detection system (Amersham). Hippocampal and cortical extracts were prepared from naïve rat brain. Two preparations were used: (i) a crude hippocampal or cortex extract was prepared by homogenizing the tissue in ice cold 320 mM sucrose in 10 mM Tris-HCl, pH 7.4; (ii) a non-denatured membrane extract was prepared by homogenizing tissue as described above, in the presence of protease inhibitors (MINI COMPLETE™ protease inhibitor, Boehringer Mannheim). Following centrifugation at 7000g, 10 min, 4°C, the resulting supernatant was centrifuged at 37,000 g, 40 min, 4°C and the pellet resuspended in 10 mM Tris-HCl, pH 7.4 containing protease inhibitors. For NMDAR1 antibody screening, 20 μ g total hippocampal extract was separated on a 12% reducing gel or 20 μ g non-denatured hippocampal membrane protein on a 10%

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Group Art Unit: 1647

Clean copy of the paragraph 2 at page 66

For the circular track mobility test, the track used was a modified version of one used to test mobility in mice (Carlsson *et al.* (1990) *Life Sci.* 47: 1729). Each rat was placed inside the track at the start position, facing clockwise, and the number of circuits completed in 5 minutes was recorded. This procedure was conducted for 5 days.

Fig. 7A depicts the results from the line crossing test and Fig. 7B depicts the results from the circular track mobility test. Data represents the number of line crossings in 5 min intervals over 5 successive days in AAVlac (squares-solid line) or AAVNMDAR1 rats (triangle-solid line). In the circular track test, the number of completed circuits in successive days for AAVlac (n=6) and AAVNMDAR1 (n=6) animals are represented.